MycoKeys 95: 209–228 (2023)
doi: 10.3897/mycokeys.95.98969
https://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

# Two new species of *Diaporthe* (Diaporthaceae, Diaporthales) in China

Ya-Quan Zhu[1], Chun-Yan Ma[2], Han Xue[1], Chun-Gen Piao[1], Yong Li[1], Ning Jiang[1]

**1** *Key Laboratory of Biodiversity Conservation of National Forestry and Grassland Administration, Ecology and Nature Conservation Institute, Chinese Academy of Forestry, Beijing 100091, China* **2** *Natural Resources and Planning Bureau of Rizhao City, Rizhao 276827, China*

**Corresponding author:** Ning Jiang (n.jiang@caf.ac.cn)

**Academic editor:** S. Maharachchikumbura | Received 18 December 2022 | Accepted 17 February 2023 | Published 1 March 2023

**Citation:** Zhu Y-Q, Ma C-Y, Xue H, Piao C-G, Li Y, Jiang N (2023) Two new species of *Diaporthe* (Diaporthaceae, Diaporthales) in China. MycoKeys 95: 209–228. https://doi.org/10.3897/mycokeys.95.98969

## Abstract

Species of *Diaporthe* have been reported as plant endophytes, pathogens and saprobes on a wide range of plant hosts. Strains of *Diaporthe* were isolated from leaf spots of *Smilax glabra* and dead culms of *Xanthium strumarium* in China, and identified based on morphology and molecular phylogenetic analyses of combined internal transcribed spacer region (ITS), calmodulin (*cal*), histone H3 (*his3*), translation elongation factor 1-alpha (*tef1*) and β-tubulin (*tub2*) loci. As a result, two new species named *Diaporthe rizhaoensis* and *D. smilacicola* are identified, described and illustrated in the present study.

## Keywords

Leaf spots, morphology, multi-gene phylogeny, taxonomy

## Introduction

*Diaporthe* (Diaporthaceae, Diaporthales) is a species-rich genus with its asexual morph previously known as *Phomopsis* (Rossman et al. 2007; Udayanga et al. 2011, 2012a, 2014a, 2015; Dissanayake et al. 2017; Guarnaccia et al. 2018). The genus *Diaporthe* was established by Nitschke in 1870 and predates its sexual morph established in 1905, thus *Diaporthe* is recommended to be used for this genus following "one fungus one name" nomenclature (Nitschke 1870; Rossman et al. 2015).

Copyright *Ya-Quan Zhu et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

The sexual morph of *Diaporthe* is characterized by immersed ascomata and an erumpent pseudostroma with single or multiple tapering perithecial necks. Asci are unitunicate, sessile and clavate to cylindrical. Ascospores are elliptical to fusiform, septate or aseptate, hyaline, biseriate to uniseriate in the ascus and sometimes have appendages (Udayanga et al. 2011; Senanayake et al. 2017, 2018). The asexual morph is characterized by black or dark brown conidiomata, with cylindrical phialides producing three types of aseptate and hyaline conidia (Type I: α-conidia, hyaline, fusiform, straight, guttulate or eguttulate, aseptate, smooth-walled; type II: β-conidia, hyaline, filiform, straight or hamate, aseptate, smooth-walled, eguttulate; type III: γ-conidia, rarely produced, hyaline, multiguttulate, fusiform to subcylindrical with an acute or rounded apex, while the base is sometimes truncate) (Udayanga et al. 2011; Gomes et al. 2013).

Species of *Diaporthe* are widely distributed, and infect a broad plant host range, e.g., agricultural crops, forest trees, vegetables, and fruits (Farr et al. 2002a, b; Crous 2005; Rossman et al. 2007; Udayanga et al. 2011, 2012a, b, 2014a, b, 2015; Gomes et al. 2013; Du et al. 2016; Dissanayake et al. 2017; Guarnaccia and Crous 2017; Fan et al. 2018). As plant pathogens, *Diaporthe* spp. cause severe diseases, e.g., blights, cankers, decay, dieback, leaf spots and wilt of many economically important plants in genera *Castanea*, *Citrus*, *Helianthus*, *Macadamia*, *Rosa*, *Vaccinium* and *Vitis*, resulting in major losses (Thompson et al. 2011; Huang et al. 2015; Guarnaccia et al. 2018, 2020; Hilário et al. 2020; Wrona et al. 2020; Caio et al. 2021; Jiang et al. 2021a).

The genus *Diaporthe* includes over 1000 epithets, mostly based on morphological characteristics and host associations (van der Aa et al. 1990; Santos et al. 2010; Guarnaccia et al. 2018). However, recent studies have shown that many species of *Diaporthe* are not host-specific, i.e., one species may infect more than one host species (Vrandecic et al. 2011; Bai et al. 2015; Zhang et al. 2018). And many *Diaporthe* species that are morphologically similar have proven to be genetically distinct (van Rensburg et al. 2006; Udayanga et al. 2011; Jiang et al. 2021b). Thus, polyphasic taxonomy is essential to identify and comprehensively characterize *Diaporthe*.

In the present study, we have analyzed five-locus dataset of combined nuclear ribosomal internal transcribed spacer (ITS), calmodulin (*cal*), histone (*his3*), translation elongation factor 1-alpha (*tef1*) and beta-tubulin (*tub2*). To aid the identification of two new species, we followed Norphanphoun et al. (2022) for the taxonomic treatments of *Diaporthe*. Norphanphoun et al. (2022) clustered *Diaporthe* into 13 workable species complexes namely *D. arecae*, *D. biconispora*, *D. carpini*, *D. decedens*, *D. eres*, *D. oncostoma*, *D. pustulata*, *D. rudis*, *D. scobina*, *D. sojae*, *D. toxica*, *D. varians* and *D. vawdreyi* species complexes. In addition, nine species were retained as singletons, viz., *D. acerina*, *D. acutispora*, *D. crataegi*, *D. multiguttulata*, *D. ocoteae*, *D. perjuncta*, *D. pseudoalnea*, *D. spartinicola* and *D. undulata* based on multilocus phylogeny.

In previous studies, *Smilax glabra* and *Xanthium strumarium* have been reported as hosts of *Diaporthe* (Vrandecic et al. 2007, 2010; Gao et al. 2013; Thompson et al. 2018). *D. eres* (= *D. mahothocarpi*) and *D. lithocarpi* were identified as the cause agents of leaf spot disease based on morphology and phylogenetics on *S. glabra* in China (Gao et al. 2013). *D. helianthi* and *D. longicolla*, pathogens of *X. strumarium*, have

been collected from blighted stems and branches in Croatia (Vrandecic et al. 2007, 2010). *D. pseudolongicolla* (= *D. novem*) has been reported as a branch dieback agent in *X. strumarium* in Australia (Thompson et al. 2018).

In this study, we introduce two new species namely *Diaporthe rizhaoensis* and *D. smilacicola*, collected from diseased plant tissues in China. We further provide descriptions, illustrations, and DNA sequence-based phylogeny to verify identification and placement.

## Materials and methods

### Isolation and morphological characterization

During 2021 and 2022, investigations were conducted to inspect for the presence of *Diaporthe* species associated with plant diseases in China. Leaves of *Smilax glabra* and culms of *Xanthium strumarium* showing typical symptoms of *Diaporthe* were collected. Infected tissues were cut into 0.5 × 0.5 cm pieces using a double-edge blade, and surface sterilized as follows. These sections underwent initial immersion for 2 min in 0.5% sodium hypochlorite, followed by 1 min in sterile distilled water, 2 min in 75% ethanol, and, finally, 1 min in sterile distilled water. The disinfected fragments were then plated onto the surface of potato dextrose agar (**PDA**; 200 g potatoes, 20 g dextrose, 20 g agar per L) and malt extract agar (**MEA**; 30 g malt extract, 5 g mycological peptone, 15 g agar per L), and incubated at 25 °C to obtain the pure culture.

Species identification was based on morphological features of the new species produced on infected plant tissues and PDA plates. Conidiomata were sectioned by hand, using a double-edged blade and structures were observed under a dissecting microscope. Over 20 fruiting bodies were sectioned, and 50 conidia were selected randomly for measurement using Axio Imager 2 microscope (Zeiss, Oberkochen, Germany). Isolate characteristics incubated on PDA at 25 °C were observed and recorded at 7 days, including colony colour, texture and the arrangement of the conidiomata. The cultures were deposited in the China Forestry Culture Collection Center (**CFCC**; http://www.cfcc-caf.org.cn/), and the specimens in the herbarium of the Chinese Academy of Forestry (**CAF**; http://museum.caf.ac.cn/).

### DNA extraction, amplification and sequencing

Genomic DNA was extracted from the fresh mycelium harvested from PDA plates after 7 days using a cetyltrimethylammonium bromide (**CTAB**) method (Doyle and Doyle 1990). For initial species confirmation, the internal transcribed spacer (**ITS**) region was sequenced for all isolates. The BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare the resulting sequences with those in GenBank. After confirmation of *Diaporthe* species, four additional partial loci, including calmodulin (*cal*), histone H3 (*his3*), partial translation elongation factor 1-alpha (*tef1*) and part of the beta-tubulin

gene region (*tub2*) genes were amplified. The primer pairs and amplification conditions for each of the above-mentioned gene regions are provided in Table 1. A PCR reaction was conducted in a 20 μL reaction volume, and the components were as follows: 1 μL DNA template (20 ng/μl), 1 μL forward 10 μM primer, 1 μL reverse 10 μM primer, 10 μL T5 Super PCR Mix (containing Taq polymerase, dNTP and $Mg^{2+}$, Beijing TisingKe Biotech Co., Ltd., Beijing, China), and 7 μL sterile water. Amplifications were performed using a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). Strands were sequenced in both directions using PCR primers. All amplified PCR products were estimated visually 1.4% agarose gels stained with ethidium bromide and then PCR positive products were sent to Sangon Biotech (Shanghai) Co., Ltd., (Beijing, China) for sequencing.

## Phylogenetic analyses

Sequences were edited and condensed with SeqMan v.7.1.0. The sequences generated in this study were supplemented with additional sequences obtained from GenBank (Table 2) based on blast searches and recent publications of the genus *Diaporthe*. The sequences were aligned with the MAFFT v.7 after which the alignments were manually corrected using MEGA v. 7.0. (Katoh and Toh 2010; Kumar et al. 2016). Phylogenetic analyses including Maximum Likelihood (**ML**) and Bayesian Inference (**BI**) methods were conducted for the single gene sequence data sets of the ITS, *cal*, *his3*, *tef1* and *tub2*, and the combined data set of all five gene regions. ML analyses were conducted using RAxML-HPC BlackBox 8.2.10 on the CIPRES Science Gateway portal (https://www.phylo.org) (Miller et al. 2012), employing a GTRGAMMA substitution model with 1000 bootstrap replicates (Stamatakis 2014). BI analyses were conducted using a Markov Chain Monte Carlo (**MCMC**) algorithm in MrBayes v.3.0 (Ronquist and Huelsenbeck 2003). Two Markov chain Monte Carlo (**MCMC**) chains were run from a random starting tree for 1,000,000 generations, resulting in a total of 10,000 trees. The first 25% of trees sampled were discarded as burn-in and the remaining trees were used to calculate the posterior probabilities. Branches with significant Bayesian Posterior Probabilities (**BPP** > 0.9) were estimated in the remaining 7,500 trees. Phylogenetic trees were viewed with FigTree v. 1.4 and processed by Adobe Illustrator CS5. The nucleotide sequence data of the new taxa were deposited in GenBank, and the GenBank accession numbers of all accessions included in the phylogenetic analyses are listed in Table 2.

**Table 1.** Loci used in this study with PCR primers and process.

| Loci | PCR primers | PCR: thermal cycles: (Annealing temp. in bold) | Reference |
|---|---|---|---|
| ITS | ITS1/ITS4 | (95 °C: 30 s, **48 °C**: 30 s, 72 °C: 1 min) × 35 cycles | White et al. 1990 |
| *cal* | CAL228F/CAL737R | (95 °C: 15 s, **54 °C**: 20 s, 72 °C: 1 min) × 35 cycles | Carbone and Kohn 1999 |
| *his3* | CYLH3F/H3-1b | (95 °C: 30 s, **57 °C**: 30 s, 72 °C: 1 min) × 35 cycles | Crous et al. 2004<br>Glass and Donaldson 1995 |
| *tef1* | EF1-728F/EF1-986R | (95 °C: 15 s, **54 °C**: 20 s, 72 °C: 1 min) × 35 cycles | Carbone and Kohn 1999 |
| *tub2* | T1(Bt2a)/Bt2b | (95 °C: 30 s, **55 °C**: 30 s, 72 °C: 1 min) × 35 cycles | Glass and Donaldson 1995<br>O'Donnell and Cigelnik 1997 |

**Table 2.** Strains and GenBank accession numbers used in this study.

| Species | Location | Host | Strain | GenBank Accession Number | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ITS | *tef1* | *tub2* | *cal* | *his3* |
| *Diaporthe absenteum* | China | *Camellia sinensis* | LC3429* | KP267897 | KP267971 | KP293477 | NA | KP293547 |
| *D. absenteum* | China | *Camellia sinensis* | LC3564 | KP267912 | KP267986 | KP293492 | NA | KP293559 |
| *D. acaciarum* | Tanzania | *Acacia tortilis* | CBS 138862* | KP004460 | NA | KP004509 | NA | KP004504 |
| *D. acericola* | Italy | *Acer negundo* | MFLUCC 17-0956* | KY964224 | KY964180 | KY964074 | KY964137 | NA |
| *D. aceris* | Japan | *Acer* sp. | LC8112 | KY491547 | KY491557 | KY491567 | KY491575 | NA |
| *D. actinidiae* | New Zealand | *Actinidia deliciosa* | ICMP 13683* | KC145886 | KC145941 | NA | NA | NA |
| *D. acuta* | China | *Pyrus pyrifolia* | CGMCC 3.19600* | MK626957 | MK654802 | MK691225 | MK691124 | MK726161 |
| *D. alangii* | China | *Alangium kurzii* | CFCC 52556* | MH121491 | MH121533 | MH121573 | MH121415 | MH121451 |
| *D. alangii* | China | *Alangium kurzii* | CFCC 52557 | MH121492 | MH121534 | MH121574 | MH121416 | MH121452 |
| *D. alnea* | Netherlands | *Alnus* sp. | CBS 146.46 | KC343008 | KC343734 | KC343976 | KC343250 | KC343492 |
| *D. amaranthophila* | Japan | *Amaranthus tricolor* | MAFF 246900 | LC459575 | LC459577 | LC459579 | LC459583 | LC459581 |
| *D. ambigua* | South Africa | *Pyrus communis* | CBS 114015* | KC343010 | KC343736 | KC343978 | KC343252 | KC343494 |
| *D. angelicae* | Austria | *Heracleum sphondylium* | CBS 111592* | KC343027 | KC343753 | KC343995 | KC343269 | KC343511 |
| *D. anhuiensis* | China | *Cunninghamia lanceolata* | CNUCC 201901* | MN219718 | MN224668 | MN227008 | MN224549 | MN224556 |
| *D. arctii* | Austria | *Arctium lappa* | CBS 139280* | KJ590736 | KJ590776 | KJ610891 | KJ612133 | KJ659218 |
| *D. arecae* | India | *Areca catechu* | CBS 161.64* | KC343032 | KC343758 | KC344000 | KC343274 | KC343516 |
| *D. arengae* | Hong Kong | *Arenga engleri* | CBS 114979* | KC343034 | KC343760 | KC344002 | KC343276 | KC343518 |
| *D. arezzoensis* | Italy | *Cytisus* sp. | MFLUCC 15-0127 | MT185503 | NA | NA | NA | NA |
| *D. aseana* | Thailand | *Unidentified dead leaf* | MFLUCC 12-0299a* | KT459414 | KT459448 | KT459432 | KT459464 | NA |
| *D. australiana* | Australia | *Macadamia* | CBS 146457 | MN708222 | MN696522 | MN696530 | NA | NA |
| *D. batatas* | USA | *Ipomoea batatas* | CBS 122.21* | KC343040 | KC343766 | KC344008 | KC343282 | KC343524 |
| *D. beilharziae* | Australia | *Indigofera australis* | BRIP 54792* | JX862529 | JX862535 | KF170921 | NA | NA |
| *D. biconispora* | China | *Citrus grandis* | ZJUD62 | KJ490597 | KJ490476 | KJ490418 | MT227578 | KJ490539 |
| *D. biguttulata* | China | *Citrus limon* | ZJUD47* | KJ490582 | KJ490461 | KJ490403 | NA | KJ490524 |
| *D. brasiliensis* | Brazil | *Aspidosperma* sp. | CBS 133183* | KC343042 | KC343768 | KC344010 | KC343284 | KC343526 |
| *D. caatingaensis* | Brazil | *Tacinga inamoena* | CBS 141542* | KY085927 | KY115603 | KY115600 | NA | KY115605 |
| *D. camelliae-oleiferae* | China | *Camellia oleifera* | HNZZ027* | MZ509555 | MZ504707 | MZ504718 | MZ504685 | MZ504696 |
| *D. caryae* | China | *Carya illinoensis* | CFCC 52563* | MH121498 | MH121540 | MH121580 | MH121422 | MH121458 |
| *D. caryae* | China | *Carya illinoensis* | CFCC 52564 | MH121499 | MH121541 | MH121581 | MH121423 | MH121459 |
| *D. cercidis* | China | *Cercis chinensis* | CFCC 52565* | MH121500 | MH121542 | MH121582 | MH121424 | MH121460 |
| *D. cercidis* | China | *Cercis chinensis* | CFCC 52566 | MH121501 | MH121543 | MH121583 | MH121425 | MH121461 |
| *D. chiangraiensis* | Thailand | *Bauhinia* sp. | MFLUCC 17-1669* | MF190119 | MF377598 | NA | NA | NA |
| *D. chrysalidocarpi* | China | *Chrysalidocarpus lutescens* | SAUCC194.35 | MT822563 | MT855760 | MT855876 | MT855646 | MT855532 |
| *D. cichorii* | Italy | *Cichorium intybus* | MFLUCC 17-1023* | KY964220 | KY964176 | KY964104 | KY964133 | NA |
| *D. cinmomi* | China | *Cinnamomum* sp. | CFCC 52569* | MH121504 | MH121546 | MH121586 | NA | MH121464 |
| *D. cinmomi* | China | *Cinnamomum* sp. | CFCC 52570 | MH121505 | MH121547 | MH121587 | NA | MH121465 |
| *D. citriasiana* | China | *Citrus unshiu* | CGMCC 3.15224* | JQ954645 | JQ954663 | KC357459 | KC357491 | KJ490515 |
| *D. columnaris* | USA | *Vaccinium vitisidaea* | AR3612* | AF439625 | NA | NA | NA | NA |
| *D. compacta* | China | *Camellia sinensis* | CGMCC 3.17536* | KP267854 | KP267928 | KP293434 | NA | KP293508 |
| *D. convolvuli* | Turkey | *Convolvulus arvensis* | CBS 124654* | KC343054 | KC343780 | KC344022 | KC343296 | KC343538 |
| *D. cucurbitae* | Canada | *Cucumis* sp. | DAOM 42078* | KM453210 | KM453211 | KP118848 | NA | KM453212 |
| *D. cuppatea* | South Africa | *Aspalathus linearis* | CBS 117499* | KC343057 | KC343783 | KC344025 | KC343299 | KC343541 |
| *D. cyatheae* | Taiwan | *Cyathea lepifera* | YMJ 1364* | JX570889 | KC465406 | KC465403 | KC465410 | NA |

| Species | Location | Host | Strain | GenBank Accession Number | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ITS | *tef1* | *tub2* | *cal* | *his3* |
| *D. discoidispora* | China | *Citrus unshiu* | ZJUD89* | KJ490624 | KJ490503 | KJ490445 | NA | KJ490566 |
| *D. drenthii* | Australia | *Macadamia* | CBS 146453 | MN708229 | MN696526 | MN696537 | NA | NA |
| *D. durionigena* | Vietnam | *Durio zibethinus* | VTCC 930005 | MN453530 | MT276157 | MT276159 | NA | NA |
| *D. endocitricola* | China | *Citrus maxima* | ZHKUCC 20-0012* | MT355682 | MT409336 | MT409290 | MT409312 | NA |
| *D. endophytica* | Brazil | *Schinus terebinthifolius* | CBS 133811* | KC343065 | KC343791 | KC344033 | KC343307 | KC343549 |
| *D. eucalyptorum* | China | *Eucalyptus* | CBS 132525* | MH305525 | NA | NA | NA | NA |
| *D. eugeniae* | Indonesia | *Eugenia aromatica* | CBS 444.82* | KC343098 | KC343824 | KC344066 | KC343340 | KC343582 |
| *D. fraxini-angustifoliae* | Australia | *Fraxinus angustifolia* | BRIP 54781* | JX862528 | JX862534 | KF170920 | NA | NA |
| *D. fructicola* | Japan | *Passiflora edulis* × *P. edulis f.* | MAFF 246408* | LC342734 | LC342735 | LC342736 | LC342738 | LC342737 |
| *D. fulvicolor* | China | *Pyrus pyrifolia* | CGMCC 3.19601* | MK626859 | MK654806 | MK691236 | MK691132 | MK726163 |
| *D. ganjae* | USA | *Cannabis sativa* | CBS 180.91* | KC343112 | KC343838 | KC344080 | KC343354 | KC343596 |
| *D. goulteri* | Australia | *Helianthus annuus* | BRIP 55657a* | KJ197290 | KJ197252 | KJ197270 | NA | NA |
| *D. guangdongensis* | China | *Citrus maxima* | ZHKUCC 20-0014* | MT355684 | MT409338 | MT409292 | MT409314 | NA |
| *D. guangxiensis* | China | *Vitis vinifera* | JZB320094* | MK335772 | MK523566 | MK500168 | MK736727 | NA |
| *D. gulyae* | Australia | *Helianthus annuus* | BRIP 54025* | JF431299 | JN645803 | KJ197271 | NA | NA |
| *D. guttulata* | China | Unknown | CGMCC 3.20100 | MT385950 | MT424685 | MT424705 | MW022470 | MW022491 |
| *D. helianthi* | Serbia | *Helianthus annuus* | CBS 592.81* | KC343115 | KC343841 | KC344083 | KC343357 | KC343599 |
| *D. heterostemmatis* | China | *Heterostemma grandiflorum* | SAUCC194.85* | MT822613 | MT855925 | MT855810 | MT855692 | MT855581 |
| *D. hongkongensis* | China | *Dichroa febrifuga* | CBS 115448* | KC343119 | KC343845 | KC344087 | KC343361 | KC343603 |
| *D. hordei* | Norway | *Hordeum vulgare* | CBS 481.92* | KC343120 | KC343846 | KC344088 | KC343362 | KC343604 |
| *D. huangshanensis* | China | *Camellia oleifera* | CNUCC 201903* | MN219729 | MN224670 | MN227010 | NA | MN224558 |
| *D. hubeiensis* | China | *Vitis vinifera* | JZB320123 | MK335809 | MK523570 | MK500148 | MK500235 | NA |
| *D. hunanensis* | China | *Camellia oleifera* | HNZZ023* | MZ509550 | MZ504702 | MZ504713 | MZ504680 | MZ504691 |
| *D. infecunda* | Brazil | *Schinus* sp. | CBS 133812* | KC343126 | KC343852 | KC344094 | KC343368 | KC343610 |
| *D. infertilis* | Suriname | *Camellia sinensis* | CBS 230.52* | KC343052 | KC343778 | KC344020 | KC343294 | KC343536 |
| *D. kochmanii* | Australia | *Helianthus annuus* | BRIP 54033* | JF431295 | JN645809 | NA | NA | NA |
| *D. kongii* | Australia | *Portulaca grandifla* | BRIP 54031* | JF431301 | JN645797 | KJ197272 | NA | NA |
| *D. krabiensis* | Thailand | marine based habitats | MFLUCC 17-2481* | MN047101 | MN433215 | MN431495 | NA | NA |
| *D. leucospermi* | Australia | *Leucospermum* sp. | CBS 111980* | JN712460 | KY435632 | KY435673 | KY435663 | KY435653 |
| *D. limonicola* | Malta | *Citrus limon* | CPC 28200* | NR_154980 | MF418501 | MF418582 | MF418256 | MF418342 |
| *D. litchiicola* | Australia | *Litchi chinensis* | BRIP 54900* | JX862533 | JX862539 | KF170925 | NA | NA |
| *D. lithocarpi* | China | *Lithocarpus glabra* | CGMCC 3.15175* | KC153104 | KC153095 | KF576311 | KF576235 | NA |
| *D. longicolla* | USA | *Glycine max* | FAU599 | KJ590728 | KJ590767 | KJ610883 | KJ612124 | KJ659188 |
| *D. longispora* | Canada | *Ribes* sp. | CBS 194.36* | KC343135 | KC343861 | KC344103 | KC343377 | KC343619 |
| *D. lusitanicae* | Portugal | *Foeniculum vulgare* | CBS 123212 | KC343136 | KC343862 | KC344104 | KC343378 | KC343620 |
| *D. lusitanicae* | Portugal | *Foeniculum vulgare* | CBS 123213* | MH863280 | KC343863 | KC344105 | KC343379 | KC343621 |
| *D. malorum* | Portugal | *Malus domestica* | CAA 734* | KY435638 | KY435627 | KY435668 | KY435658 | KY435648 |
| *D. manihotia* | Rwanda | *Manihot utilissima* | CBS 505.76 | KC343138 | KC343864 | KC344106 | KC343380 | KC343622 |
| *D. masirevicii* | Australia | *Helianthus annuus* | BRIP 57892a* | KJ197276 | KJ197239 | KJ197257 | NA | NA |
| *D. mayteni* | Brazil | *Maytenus ilicifolia* | CBS 133185 | KC343139 | KC343865 | KC344107 | KC343381 | KC343623 |
| *D. megalospora* | Not stated | *Sambucus canadensis* | CBS 143.27* | KC343140 | KC343866 | KC344108 | KC343382 | KC343624 |
| *D. melitensis* | Malta | *Citrus limon* | CPC 27873* | MF418424 | MF418503 | MF418584 | MF418258 | MF418344 |
| *D. melonis* | USA | *Cucumis melo* | CBS 507.78* | KC343142 | KC343868 | KC344110 | KC343384 | KC343626 |
| *D. melonis* | Indonesia | *Glycine soja* | CBS 435.87 | KC343141 | KC343867 | KC344109 | KC343383 | KC343625 |
| *D. middletonii* | Australia | *Rapistrum rugostrum* | BRIP 54884e* | KJ197286 | KJ197248 | KJ197266 | NA | NA |
| *D. millettiae* | China | *Millettia reticulata* | GUCC9167* | MK398674 | MK480609 | MK502089 | MK502086 | NA |
| *D. minusculata* | China | saprobic on decaying wood | CGMCC 3.20098* | MT385957 | MT424692 | MT424712 | MW022475 | MW022499 |

| Species | Location | Host | Strain | GenBank Accession Number | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ITS | *tef1* | *tub2* | *cal* | *his3* |
| *D. miriciae* | Australia | *Helianthus annuus* | BRIP 54736j* | KJ197282 | KJ197244 | KJ197262 | NA | NA |
| *D. musigena* | Australia | *Musa* sp. | CBS 129519* | KC343143 | KC343869 | KC344111 | KC343385 | KC343267 |
| *D. myracrodruonis* | Brazil | *Astronium urundeuva* | URM 7972* | MK205289 | MK213408 | MK205291 | MK205290 | 17 |
| *D. nelumbonis* | Taiwan | *Nelumbo nucifera* | R. Kirschner 4114* | KT821501 | NA | LC086652 | NA | NA |
| *D. neoarctii* | USA | *Ambrosia trifi* | CBS 109490* | KC343145 | KC343871 | KC344113 | KC343387 | KC343629 |
| *D. neoraonikayaporum* | Thailand | *Tectona grandis* | MFLUCC 14-1136* | KU712449 | KU749369 | KU743988 | KU749356 | NA |
| *D. oculi* | Japan | *Homo sapiens* | HHUF 30565* | LC373514 | LC373516 | LC373518 | NA | NA |
| *D. osmanthi* | China | *Osmanthus fragrans* | GUCC9165* | MK398675 | MK480610 | MK502091 | MK502087 | NA |
| *D. ovalispora* | China | *Citrus limon* | CGMCC 3.17256* | KJ490628 | KJ490507 | KJ490449 | NA | KJ490570 |
| *D. oxe* | Brazil | *Maytenus ilicifolia* | CBS 133186* | KC343164 | KC343890 | KC344132 | KC343406 | KC343648 |
| *D. pandanicola* | Thailand | *Pandanus* sp. | MFLUCC 17-0607* | MG646974 | NA | MG646930 | NA | NA |
| *D. paranensis* | Brazil | *Maytenus ilicifolia* | CBS 133184* | KC343171 | KC343897 | KC344139 | KC343413 | KC343655 |
| *D. pascoei* | Australia | *Persea americana* | BRIP 54847* | JX862532 | JX862538 | KF170924 | NA | NA |
| *D. passiflorae* | South America | *Passiflora edulis* | CBS 132527* | JX069860 | KY435633 | KY435674 | KY435664 | KY435654 |
| *D. passifloricola* | Malaysia | *Passiflora foetida* | CBS 141329* | KX228292 | NA | KX228387 | NA | KX228367 |
| *D. perseae* | Netherlands | *Persea gratissima* | CBS 151.73* | KC343173 | KC343899 | KC343141 | KC343415 | KC343657 |
| *D. pescicola* | China | *Prunus persica* | MFLUCC 16-0105* | KU557555 | KU557623 | KU557579 | KU557603 | NA |
| *D. phaseolorum* | USA | *Phaseolus vulgaris* | AR4203* | KJ590738 | KJ590739 | KJ610893 | KJ612135 | KJ659220 |
| *D. phoenicicola* | India | *Areca catechu* | CBS 161.64* | MH858400 | GQ250349 | JX275440 | JX197432 | NA |
| *D. podocarpi-macrophylli* | China | *Podocarpus macrophyllus* | CGMCC 3.18281* | KX986774 | KX999167 | KX999207 | KX999278 | KX999246 |
| *D. pseudolongicolla* | Serbia | *Glycine max* | PL42* | JQ697843 | JQ697856 | NA | NA | NA |
| *D. pseudolongicolla* | Croatia | *Glycine max* | CBS 127269 | KC343155 | KC343881 | KC344123 | KC343397 | KC343639 |
| *D. pseudomangiferae* | Dominican Republic | *Mangifera indica* | CBS 101339* | KC343181 | KC343907 | KC344149 | KC343423 | KC343665 |
| *D. pseudooculi* | Japan | *Homo sapiens* | HHUF 30617* | NR_161019 | LC373517 | LC373519 | NA | NA |
| *D. pseudophoenicicola* | Spain | *Phoenix dactylifera* | CBS 462.69* | KC343184 | KC343910 | KC344152 | KC343426 | KC343668 |
| *D. pseudophoenicicola* | Iraq | *Mangifera indica* | CBS 176.77 | KC343183 | KC343909 | KC344151 | KC343425 | KC343667 |
| *D. pterocarpicola* | Thailand | *Pterocarpus indicus* | MFLUCC 10-0580a* | JQ619887 | JX275403 | JX275441 | JX197433 | NA |
| *D. pyracanthae* | Portugal | *Pyracantha coccinea* | CBS 142384* | KY435635 | KY435625 | KY435666 | KY435656 | KY435646 |
| *D. racemosae* | South Africa | *Euclea racemosa* | CPC 26646* | MG600223 | MG600225 | MG600227 | MG600219 | MG600221 |
| *D. raonikayaporum* | Brazil | *Spondias mombin* | CBS 133182* | KC343188 | KC343914 | KC344156 | KC343430 | KC343672 |
| *D. rhodomyrti* | China | *Rhodomyrtus tomentosa* | CFCC 53101 | MK432643 | MK578119 | MK578046 | MK442965 | MK442990 |
| *D. rhodomyrti* | China | *Rhodomyrtus tomentosa* | CFCC 53102 | MK432644 | MK578120 | MK578047 | MK442966 | MK442991 |
| ***D. rizhaoensis*** | **China** | ***Xanthium strumarium*** | **CFCC 57562*** | **OP955930** | **OP959767** | **OP959773** | **OP959782** | **OP959785** |
| ***D. rizhaoensis*** | **China** | ***Xanthium strumarium*** | **CFCC 57563** | **OP955931** | **OP959766** | **OP959772** | **OP959781** | **OP959784** |
| ***D. rizhaoensis*** | **China** | ***Xanthium strumarium*** | **CFCC 57564** | **OP955932** | **OP959765** | **OP959771** | **OP959780** | **OP959783** |
| *D. rosae* | Thailand | *Rosa* sp. | MFLUCC 17-2658* | MG828894 | NA | MG843878 | MG829273 | NA |
| *D. rosiphthora* | Brazil | *Rosa* sp. | COAD 2914* | MT311197 | MT313693 | NA | MT313691 | NA |
| *D. rossmaniae* | Portugal | *Vaccinium corymbosum* | CAA762* | MK792290 | MK828063 | MK837914 | MK883822 | MK871432 |
| *D. sackstonii* | Australia | *Helianthus annuus* | BRIP 54669b* | KJ197287 | KJ197249 | KJ197267 | NA | NA |
| *D. salinicola* | Thailand | *Xylocarpus* sp. | MFLU 18-0553* | MN047098 | MN077073 | NA | NA | NA |

| Species | Location | Host | Strain | GenBank Accession Number | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ITS | *tef1* | *tub2* | *cal* | *his3* |
| *D. sambucusii* | China | *Sambucus williamsii* | CFCC 51986* | KY852495 | KY852507 | KY852511 | KY852499 | KY852503 |
| *D. sambucusii* | China | *Sambucus williamsii* | CFCC 51987 | KY852496 | KY852508 | KY852512 | KY852500 | KY852504 |
| *D. schimae* | China | *Schima superba* | CFCC 53103* | MK432640 | MK578116 | MK578043 | MK442962 | MK442987 |
| *D. schimae* | China | *Schima superba* | CFCC 53104 | MK432641 | MK578117 | MK578044 | MK442963 | MK442988 |
| *D. schini* | Brazil | *Schinus terebinthifolius* | CBS 133181* | KC343191 | KC343917 | KC344159 | KC343433 | KC343675 |
| *D. schoeni* | Italy | *Schoenus nigricans* | MFLU 15-1279* | KY964226 | KY964182 | KY964109 | KY964139 | |
| *D. sclerotioides* | Netherlands | *Cucumis sativus* | CBS 296.67* | KC343193 | KC343919 | KC344161 | KC343435 | KC343677 |
| *D. searlei* | Australia | *Macadamia* | CBS 146456* | MN708231 | NA | MN696540 | NA | NA |
| *D. sennae* | China | *Senna bicapsularis* | CFCC 51636* | KY203724 | KY228885 | KY228891 | KY228875 | NA |
| *D. sennae* | China | *Senna bicapsularis* | CFCC 51637 | KY203725 | KY228886 | KY228892 | KY228876 | NA |
| *D. serafiniae* | Australia | *Helianthus annuus* | BRIP 55665a* | KJ197274 | KJ197236 | KJ197254 | NA | NA |
| *D. siamensis* | Thailand | *Dasymaschalon* sp. | MFLUCC 10-0573a* | JQ619879 | JX275393 | JX275429 | JX197423 | NA |
| *D. sinensis* | China | *Amaranthus* sp. | ZJUP0033-4* | MK637451 | MK660449 | MK660447 | NA | MK660451 |
| ***D. smilacicola*** | **China** | ***Smilax glabra*** | **CFCC 54582*** | **OP955933** | **OP959770** | **OP959776** | **OP959779** | **OP959788** |
| ***D. smilacicola*** | **China** | ***Smilax glabra*** | **CFCC 58764** | **OP955934** | **OP959769** | **OP959775** | **OP959778** | **OP959787** |
| ***D. smilacicola*** | **China** | ***Smilax glabra*** | **CFCC 58765** | **OP955935** | **OP959768** | **OP959774** | **OP959776** | **OP959786** |
| *D. sojae* | USA | *Glycine max* | FAU635* | KJ590719 | KJ590762 | KJ610875 | KJ612116 | KJ659208 |
| *D. spinosa* | China | *Pyrus pyrifolia* | CGMCC 3.19602* | MK626849 | MK654811 | MK691234 | MK691129 | MK726156 |
| *D. stewartii* | Not stated | *Cosmos bipinnatus* | CBS 193.36* | MH867279 | GQ250324 | JX275421 | JX197415 | NA |
| *D. subellipicola* | China | on dead wood | KUMCC 17-0153* | MG746632 | MG746633 | MG746634 | NA | NA |
| *D. subordinaria* | New Zealand | *Plantago lanceolata* | CBS 464.90* | KC343214 | KC343940 | KC344182 | KC343456 | KC343698 |
| *D. taiwanensis* | Taiwan | *Ixora chinensis* | NTUCC 18-105-1* | MT241257 | MT251199 | MT251202 | MT251196 | NA |
| *D. taoicola* | China | *Prunus persica* | MFLUCC 16-0117* | KU557567 | KU557635 | KU557591 | NA | NA |
| *D. tarchonanthi* | South Africa | *Tarchonanthus littoralis* | CBS 146073* | MT223794 | NA | MT223733 | NA | MT223759 |
| *D. tecomae* | Brazil | *Tabebuia* sp. | CBS 100547* | KC343215 | KC343941 | KC344183 | KC343457 | KC343699 |
| *D. tectonae* | Thailand | *Tectona grandis* | MFLUCC 12-0777* | KU712430 | KU749359 | KU743977 | KU749345 | NA |
| *D. tectonendophytica* | Thailand | *Tectona grandis* | MFLUCC 13-0471* | KU712439 | KU749367 | KU743986 | KU749354 | NA |
| *D. tectonigena* | China | *Tectona grandis* | MFLUCC 12-0767* | KU712429 | KU749371 | KU743976 | KU749358 | NA |
| *D. tectonigena* | China | *Camellia sinensis* | LC6512 | KX986782 | KX999174 | KX999214 | KX999284 | KX999254 |
| *D. terebinthifolii* | Brazil | *Schinus terebinthifolius* | CBS 133180* | KC343216 | KC343942 | KC344184 | KC343458 | KC343700 |
| *D. thunbergiicola* | Thailand | *Thunbergia laurifolia* | MFLUCC 12-0033* | KP715097 | KP715098 | NA | NA | NA |
| *D. tulliensis* | Australia | *Theobroma cacao* | BRIP 62248a* | KR936130 | KR936133 | KR936132 | NA | NA |
| *D. ueckeri* | USA | *Cucumis melo* | FAU656* | KJ590726 | KJ590747 | KJ610881 | KJ612122 | KJ659215 |
| *D. unshiuensis* | China | *Fortunella margarita* | CGMCC 3.17566* | KJ490584 | KJ490463 | KJ490405 | NA | KJ490526 |
| *D. unshiuensis* | China | *Carya illinoensis* | CFCC 52594 | MH121529 | MH121571 | MH121606 | MH121447 | MH121487 |
| *D. unshiuensis* | China | *Carya illinoensis* | CFCC 52595 | MH121530 | MH121572 | MH121607 | MH121448 | MH121488 |
| *D. vawdreyi* | Australia | *Psidium guajava* | BRIP 57887a | KR936126 | KR936129 | KR936128 | NA | NA |
| *D. vexans* | USA | *Solanum melongena* | CBS 127.14 | KC343229 | KC343955 | KC344197 | KC343471 | KC343713 |
| *D. viniferae* | China | *Vitis vinifera* | JZB320071* | MK341550 | MK500107 | MK500112 | MK500119 | NA |
| *D. vochysiae* | Brazil | *Vochysia divergens* | LGMF1583* | MG976391 | MK007526 | MK007527 | MK007528 | MK033323 |
| *D. xishuangbanica* | China | *Camellia sinensis* | CGMCC 3.18283* | KX986784 | KX999176 | KX999217 | NA | NA |
| *D. xishuangbanica* | China | *Camellia sinensis* | LC6707 | KX986783 | KX999175 | KX999216 | NA | KX999255 |

Notes: NA, not applicable. * ex-type strains.

# Results

## Phylogeny

In the present study, we followed Norphanphoun et al. (2022) for the species complexes treatments of *Diaporthe*. Firstly, we conducted a genus tree including all species belonging to this genus according to Norphanphoun et al. (2022). After that, the phylogenetic analysis revealed that three isolates (CFCC 57562, CFCC 57563 and CFCC 57564) clustered in a distinct clade in the *D. sojae* species complex, and three isolates (CFCC 54582, CFCC 58764 and CFCC 58765) clustered in a distinct clade in the *D. arecae* species complex (Figs 1, 2). The combined sequence alignments of *D. arecae* species complex comprised 62 strains, with *D. vawdreyi* (BRIP 57887a) and *D. biconispora* (ZJUD62) as the outgroup taxa. The dataset comprised 2791 characters including alignment gaps (634 for ITS, 381 for *tef1*, 791 for *tub2*, 499 for *cal* and 486 for *his3*). The combined sequence alignments of *D. sojae* species complex comprised 111 strains, with *D. aceris* (LC8112) and *D. alnea* (CBS 146.46) as the outgroup taxa. The dataset comprised 2799 characters including alignment gaps (671 for ITS, 483 for *tef1*, 483 for *tub2*, 593 for *cal* and 569 for *his3*). The final maximum likelihood tree topology was similar to Bayesian analysis.

## Taxonomy

***Diaporthe rizhaoensis*** **Y.Q. Zhu & Ning Jiang, sp. nov.**
MycoBank No: 846816
Fig. 3

**Etymology.** Named after the collection site of the type specimen, Rizhao City.

**Description.** ***Conidiomata*** pycnidial, small, scattered, slightly erumpent through bark surface, nearly flat, discoid, with a solitary undivided locule, 150–400 µm diam. ***Conidiogenous cells*** 6.7–11.4 × 1.6–3.0 µm, hyaline, unbranched, densely aggregated, mostly ampulliform, guttulate, aseptate, straight or slightly curved, swelling at base, tapering towards apex. ***Beta conidia*** 12.9–23.4 × 1.1–2.1 µm (mean = 18.7 × 1.4 µm, n = 50), hyaline, filiform, straight or slightly curved, aseptate, base subtruncate, tapering towards the base. ***Alpha conidia and gamma conidia*** not observed. ***Sexual morph*** not observed.

**Culture characters.** Colonies on potato dextrose agar (PDA) flat, spreading, with flocculent aerial mycelium and entire edge, white, reaching a 90 mm diameter after 14 days at 25 °C; on malt extract agar (MEA) flat, spreading, with flocculent aerial mycelium and crenate edge, white, reaching a 90 mm diameter after 14 days at 25 °C, forming black conidiomata with black conidial masses; on synthetic low nutrient agar (SNA) flat, spreading, with flocculent aerial mycelium forming concentric rings and entire edge, white, reaching a 90 mm diameter after 14 days at 25 °C.

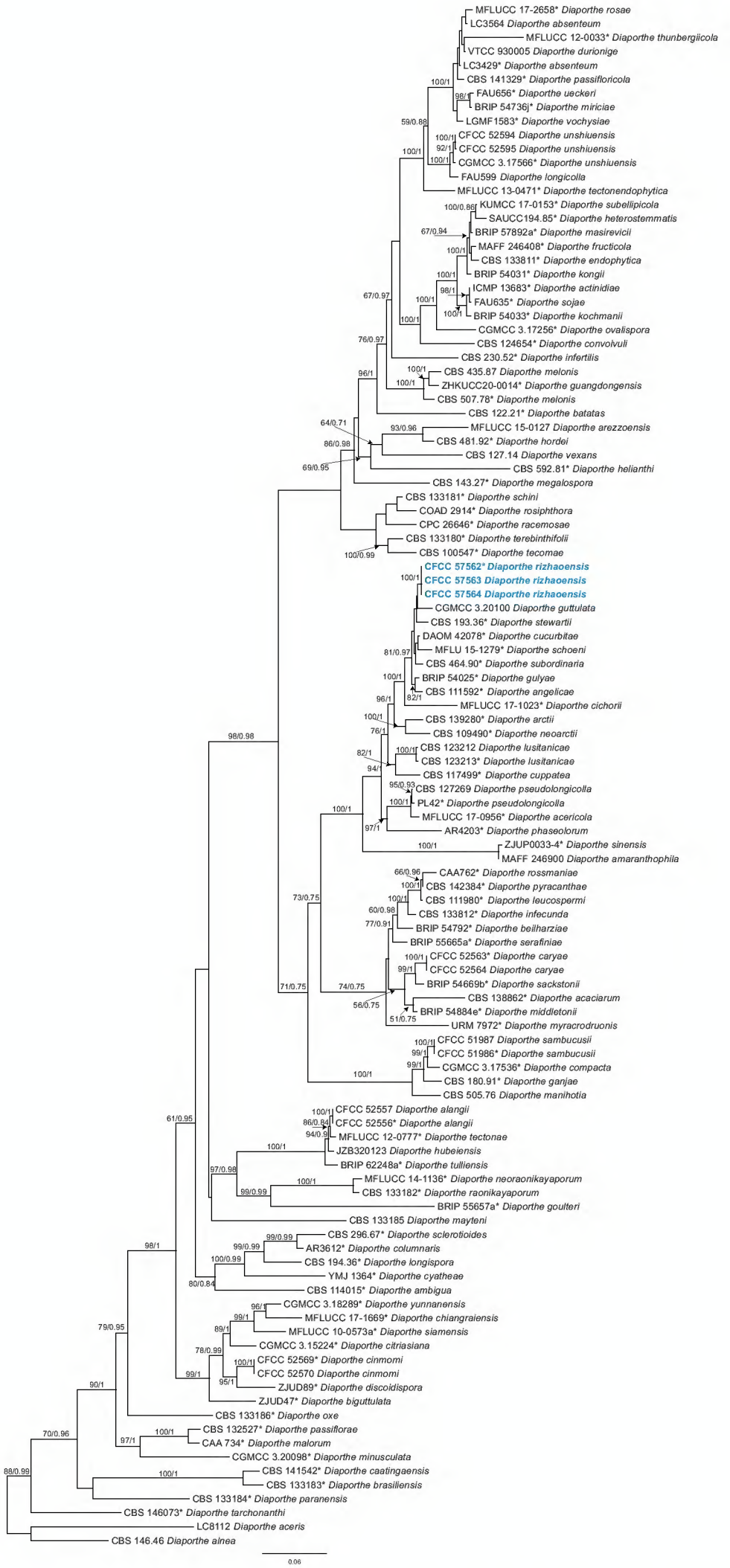


**Figure 1.** Phylogram of *Diaporthe sojae* species complex resulting from a maximum likelihood analysis based on a combined matrix of ITS, *cal*, *his3*, *tef1* and *tub2* loci. Numbers above the branches indicate ML bootstrap values (left, ML BS ≥ 50%) and Bayesian posterior probabilities (right, BPP ≥ 0.9). Isolates from the present study are marked in bold blue.

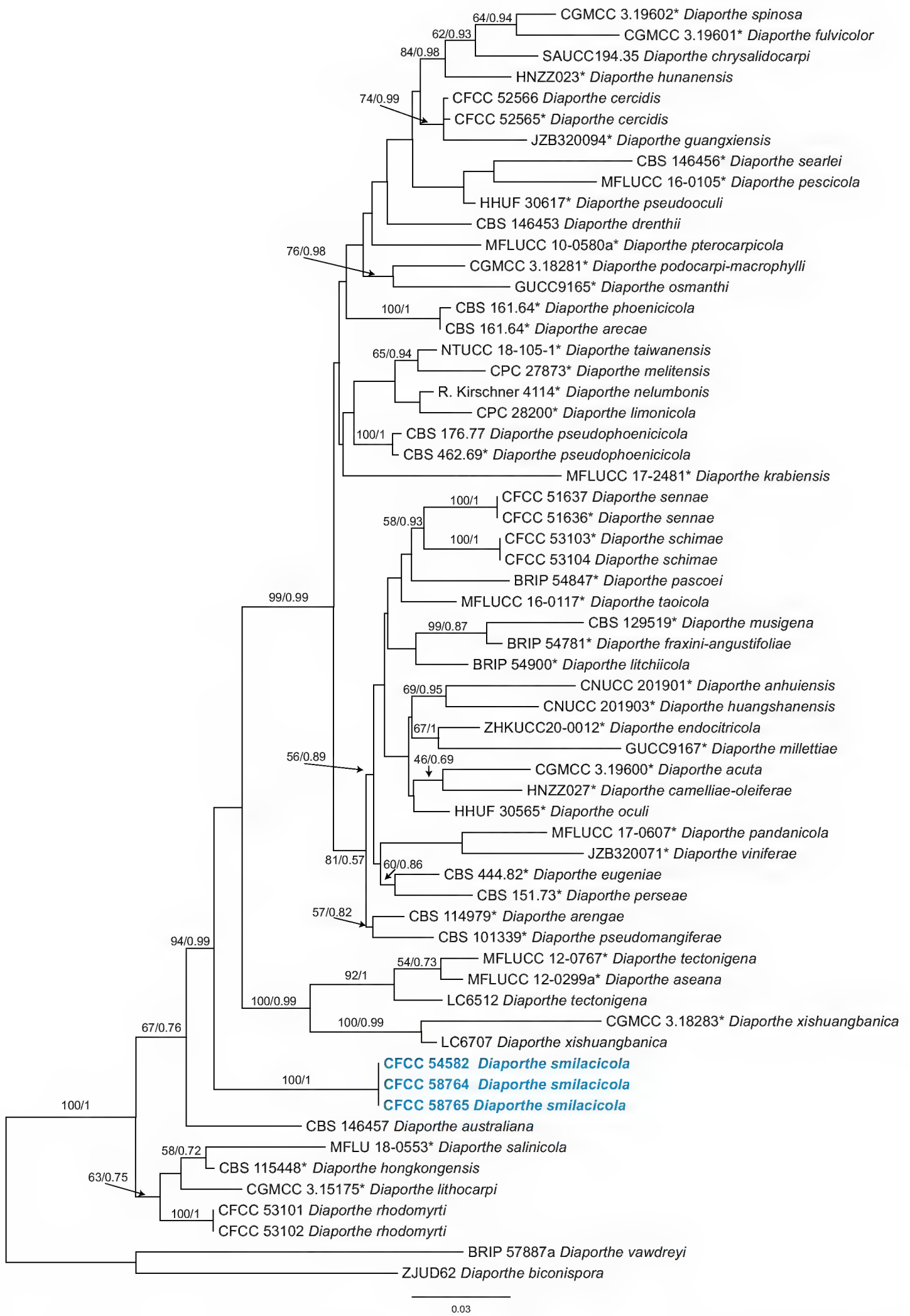


**Figure 2.** Phylogram of *Diaporthe arecae* species complex resulting from a maximum likelihood analysis based on a combined matrix of ITS, *cal*, *his3*, *tef1* and *tub2* loci. Numbers above the branches indicate ML bootstrap values (left, ML BS ≥ 50%) and Bayesian posterior probabilities (right, BPP ≥ 0.9). Isolates from the present study are marked in bold blue.

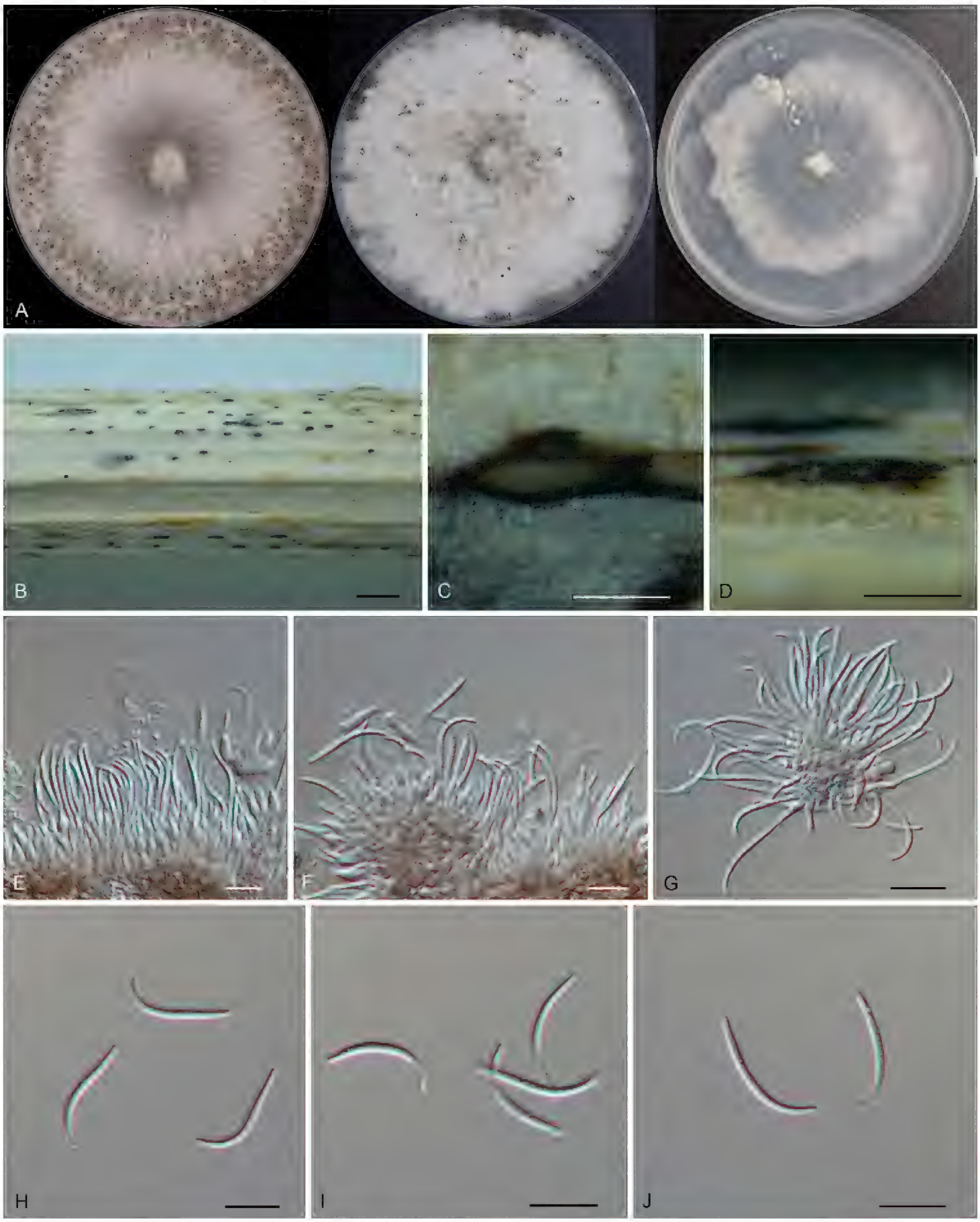


**Figure 3.** Morphology of *Diaporthe rizhaoensis* **A** colonies on PDA, MEA and SNA at 25 °C after 2 weeks **B** habit of conidiomata on the host **C** transverse section of the conidioma **D** longitudinal section through the conidioma **E–G** conidiogenous cells with attached beta conidia **H–J** beta conidia. Scale bars: 500 μm (**B**); 100 μm (**C, D**); 10 μm (**E–J**).

**Materials examined.** China, Shandong Province, Rizhao City, Wulian County, Zhongzhi Town, on dead culms of *Xanthium strumarium*, 5 May 2022, Ning Jiang & Chengbin Wang (holotype CAF 800069; ex-holotype culture CFCC 57562).

Shandong Province, Rizhao City, Wulian County, Xumeng Town, on dead culms of *Xanthium strumarium*, 5 May 2022, Ning Jiang & Chengbin Wang (cultures CFCC 57563 and CFCC 57564).

**Notes.** *Diaporthe rizhaoensis* formed a distinct clade with high support (ML/BI = 100/1), and was close to *D. guttulata* and *D. stewartia* (Fig. 1). *Diaporthe rizhaoensis* is different from *D. stewartia* by host association (*D. rizhaoensis* on *Xanthium strumarium* vs. *D. stewartia* on *Cosmos bipinnatus*) (Harrison 1935; Dissanayake et al. 2020). In addition, *D. guttulata* and *D. stewartia* are only known in sexual morph. Moreover, *Diaporthe rizhaoensis* can be distinguished from *D. guttulata* (15/364 in *cal*, 5/428 in *his3*, 5/313 in *tef1*, and 1/408 in *tub2*) and *D. stewartii* (3/532 in ITS, 7/451 in *cal*, and 7/369 in *tub2*) by sequence data. *Diaporthe helianthi*, *D. longicolla*, *D. pseudolongicolla* (= *D. novem*) and *D. rizhaoensis* have been reported form the host *Xanthium strumarium* (Vrandecic et al. 2007, 2010; Petrović et al. 2018; Thompson et al. 2018). Morphologically, *Diaporthe helianthi* is a bit longer than *D. rizhaoensis* in the beta conidia, but not fully distinguished (Vrandecic et al. 2007, 2010). Morphology of *D. longicolla* and *D. pseudolongicolla* on *Xanthium strumarium* were not available. However, these four species are phylogenetically distinguished in the phylogram of *D. sojae* species complex (Fig. 1).

## ***Diaporthe smilacicola*** **Y.Q. Zhu & Ning Jiang, sp. nov.**

MycoBank No: 846818
Fig. 4

**Etymology.** Named after the host genus, *Smilax*.

**Description.** ***Leaf spots*** subcircular to irregular, pale brown to brown, with dark brown margin. ***Conidiomata*** pycnidial, scattered, subglobose to globose, black, erumpent, exuding faint yellow translucent conidial droplets from central ostioles, 150–350 µm diam. ***Conidiogenous cells*** 11–16.2 × 1.8–2.4 µm, hyaline, phialidic, cylindrical, terminal, slightly tapering towards the apex. ***Alpha conidia*** 5.7–9.7 × 2.0–3.5 µm (mean = 7.8 × 2.6 µm, n = 50), hyaline, aseptate, smooth, guttulate, ellipsoidal to oblong ellipsoidal, with both ends obtuse. ***Beta conidia and gamma conidia*** not observed. ***Sexual morph*** not observed.

**Culture characters.** Colonies on PDA flat, with flocculent aerial mycelium and crenate edge, white to gray, reaching a 90 mm diameter after 14 days at 25 °C, forming black conidiomata with black conidial masses; on MEA flat, spreading, with flocculent aerial mycelium forming concentric rings, off-white to luteous, reaching a 90 mm diameter after 14 days at 25 °C; on SNA flat, spreading, with flocculent aerial mycelium forming concentric rings and entire edge, white, reaching a 90 mm diameter after 14 days at 25 °C.

**Materials examined.** China, Hunan Province, Changsha City, Changsha County, Kaihui Town, on leaf spots of *Smilax glabra*, 2 November 2020, Ning Jiang (holotype CAF 800070; ex-holotype culture CFCC 54582). Hunan Province, Shaoshan City, on leaf spots of *Smilax glabra*, 2 November 2020, Ning Jiang (cultures CFCC 58764 and CFCC 58765).

**Notes.** Three *Diaporthe* isolates representing *D. smilacicola* formed a well-supported clade (ML/BI = 100/1), and appear to be distinct from the other *Diaporthe* species phylogenetically (Fig. 2). *Diaporthe eres* (= *D. mahothocarpi*), *D. lithocarpi* and *D. smilacicola* have been reported from the host *S. glabra* (Gao et al. 2013; Chaisiri et al. 2021). Morphologically, these three species are similar in conidial shape and size. However, *Diaporthe eres* belongs to *D. eres* species complex, which is different from *D. lithocarpi* and *D. smilacicola* in *D. arecae* species complex. *D. smilacicola* is obviously different from *D. lithocarpi* based on sequence data (22/467 in ITS, 31/393 in *cal*, 52/317 in *tef1*, 19/420 in *tub2*) (Fig. 2).

## Discussion

Based on the morphology and the multi-locus phylogeny, six isolates from the present study can be recognized as two new species of *Diaporthe*, viz. *D. rizhaoensis* from dead culms of *Xanthium strumarium* and *D. smilacicola* from leaf spots of *Smilax glabra*.

Species identification in *Diaporthe* was primarily based on the assumption of host-specificity, which has largely impeded the progress of establishing a proper taxonomy of *Diaporthe* (Gomes et al. 2013). More than one species of *Diaporthe* can often be recovered from a single host and one species was found to be associated with different host plants (Gomes et al. 2013; Gao et al. 2017; Guarnaccia and Crous 2017; Guarnaccia et al. 2018; Guo et al. 2020). For example, *D. eres* can infect blackberry (Vrandecic et al. 2011), pear (Bai et al. 2015), and jujube (Zhang et al. 2018); *D. pometiae* was isolated from *Heliconia metallica* and *Persea americana* (Huang et al 2021); *D. melastomatis* was collected from three hosts namely *Camellia sinensis*, *Melastoma malabathricum* and *Millettia reticulata* (Sun et al. 2021); *D. australiana*, *D. drenthii*, *D. macadamiae* and *D. searlei* can cause diseases on macadamia in Australia and South Africa (Wrona et al. 2020) and seven endophytic *Diaporthe* species were discovered on *Citrus* trees (Huang et al. 2015). As was revealed in the present study, two additional species of *Diaporthe* were proposed from the host *Smilax glabra* and *Xanthium strumarium*. This study further demonstrates that host association is not a robust character to distinguish members of *Diaporthe*.

Recently, the species classification of *Diaporthe* has become more dependent on DNA sequence-based methods rather than traditional morphological characterization. (Udayanga et al. 2014a, b, 2015; Fan et al. 2015; Gao et al. 2017; Guarnaccia and Crous 2017; Guarnaccia et al. 2018; Hyde et al. 2018, 2020; Yang et al. 2018, 2020, 2021; Long et al. 2019; Cao et al. 2022). The ITS sequence offers convincing proof for species demarcation and is recommended for identifying species boundaries in the genus *Diaporthe* (Santos and Phillips 2009, 2011; Thompson et al. 2011). However, the intraspecific variation is even greater than the interspecific variation, which makes it difficult to identify *Diaporthe* species using the ITS sequence alone (Crouch et al. 2009). Considering this, concatenation of a five-loci dataset (ITS-*tef1*-*tub2*-*cal*-*his3*) was recommended as the best combination for species identification within the genus (Udayanga et al. 2014; Fan et al. 2018; Yang et al. 2018; Guo et al. 2020).

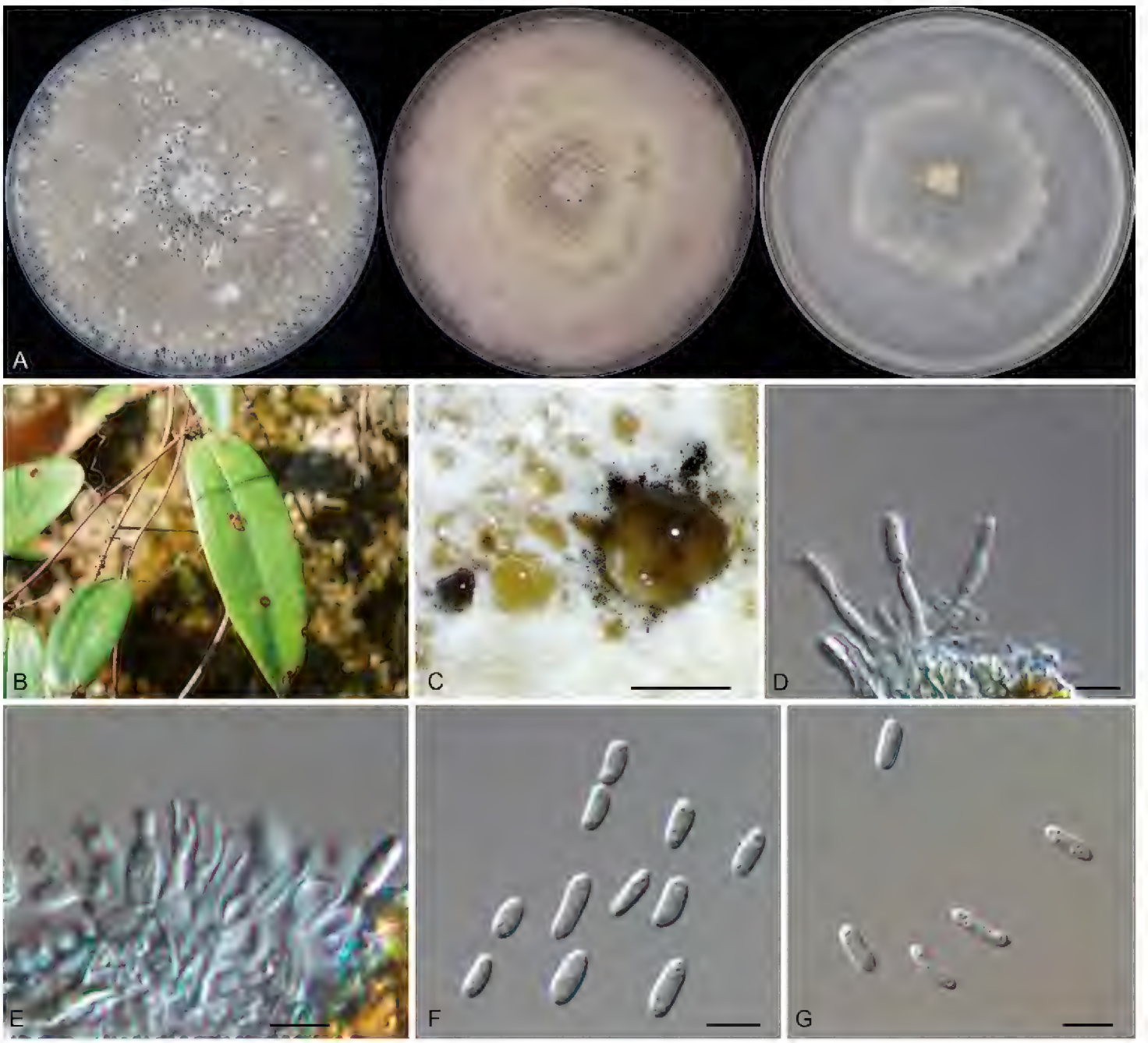


**Figure 4.** Morphology of *Diaporthe smilacicola* **A** colonies on PDA, MEA and SNA at 25 °C after 2 weeks **B** leaf spots on the host surface **C** conidiomata formed on the PDA **D, E** conidiogenous cells with attached alpha conidia **F–G** alpha conidia. Scale bars: 200 μm (**C**); 10 μm (**D–G**).

Two phylograms resulted from the present study also support the feasibility of the five loci data to separate species of *Diaporthe*.

The two newly introducing species could potentially be pathogens, because they were isolated from diseased plant tissues, and their pathogenicity should be evaluated in further studies. And, it is necessary to evaluate the effects of environmental conditions, such as temperature, pH, and carbon sources, on mycelium growth and pathogenicity.

## Acknowledgements

This research was funded by Fundamental Research Funds for the Central Non-profit Research Institution of Chinese Academy of Forestry (grant CAFYBB2018ZB001), and National Microbial Resource Center of the Ministry of Science and Technology of the People's Republic of China (grant NMRC-2021-7).

## Reference

Bai Q, Zhai LF, Chen XR, Hong N, Xu WX, Wang GP (2015) Biological and molecular characterization of five *Phomopsis* species associated with pear shoot canker in China. Plant Disease 99(12): 1704–1712. https://doi.org/10.1094/PDIS-03-15-0259-RE

Caio P, Bruno F, Carlos AP, Robert B (2021) *Diaporthe rosiphthora* sp. nov.: Yet another rose dieback fungus. Crop Protection (Guildford, Surrey) 139: 105365. https://doi.org/10.1016/j.cropro.2020.105365

Cao L, Luo D, Lin W, Yang Q, Deng X (2022) Four new species of *Diaporthe* (Diaporthaceae, Diaporthales) from forest plants in China. MycoKeys 91: 25–47. https://doi.org/10.3897/mycokeys.91.84970

Carbone I, Kohn LM (1999) A Method for designing primer sets for speciation studies in filamentous Ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/00275514.1999.12061051

Chaisiri C, Liu X, Lin Y, Fu Y, Zhu F, Luo C (2021) Phylogenetic and haplotype network analyses of *Diaporthe eres* species in China based on sequences of multiple loci. Biology (Basel) 10(3): 179. https://doi.org/10.3390/biology10030179

Crouch JA, Clarke BB, Hillman BI (2009) What is the value of ITS sequence data in *Colletotrichum* systematics and species diagnosis? A case study using the falcate-spored graminicolous *Colletotrichum* group. Mycologia 101(5): 648–656. https://doi.org/10.3852/08-231

Crous PW (2005) Impact of molecular phylogenetics on the taxonomy and diagnostics of fungi. Bulletin OEPP. EPPO Bulletin. European and Mediterranean Plant Protection Organisation 35(1): 47–51. https://doi.org/10.1111/j.1365-2338.2005.00811.x

Crous PW, Groenewald JZ, Risède JM, Simoneau P, Hywel-Jones NL (2004) *Calonectria* species and their *Cylindrocladium* anamorphs: Species with sphaeropedunculate vesicles. Studies in Mycology 50: 415–430.

Dissanayake AJ, Phillips AJL, Hyde KD, Yan JY, Li XH (2017) The current status of species in *Diaporthe*. Mycosphere 8(5): 1106–1156. https://doi.org/10.5943/mycosphere/8/5/5

Dissanayake AJ, Chen YY, Liu JK (2020) Unravelling *Diaporthe* species associated with woody hosts from karst formations (Guizhou) in China. Journal of Fungi (Basel, Switzerland) 6(4): 251. https://doi.org/10.3390/jof6040251

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus (San Francisco, Calif.) 12: 13–15.

Du Z, Fan XL, Hyde KD, Yang Q, Liang YM, Tian CM (2016) Phylogeny and morphology reveal two new species of *Diaporthe* from *Betula* spp. in China. Phytotaxa 269(2): 90–102. https://doi.org/10.11646/phytotaxa.269.2.2

Fan XL, Hyde KD, Udayanga D, Wu XY, Tian CM (2015) *Diaporthe rostrata*, a novel ascomycete from *Juglans mandshurica* associated with walnut dieback. Mycological Progress 14(10): 1–8. https://doi.org/10.1007/s11557-015-1104-5

Fan XL, Yang Q, Bezerra JDP, Alvarez LV, Tian CM (2018) *Diaporthe* from walnut tree (*Juglans regia*) in China, with insight of *Diaporthe eres* complex. Mycological Progress 17(7): 841–853. https://doi.org/10.1007/s11557-018-1395-4

Farr DF, Castlebury LA, Rossman AY (2002a) Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the eastern United States. Mycologia 94(3): 494–504. https://doi.org/10.1080/15572536.2003.11833214

Farr DF, Castlebury LA, Rossman AY, Putnam ML (2002b) A new species of *Phomopsis* causing twig dieback of *Vaccinium vitisidaea* (lingonberry). Mycological Research 106(6): 745–752. https://doi.org/10.1017/S095375620200583X

Gao YH, Sun W, Su YY, Cai L (2013) Three new species of *Phomopsis* in Gutianshan nature reserve in China. Mycological Progress 13(1): 111–121. https://doi.org/10.1007/s11557-013-0898-2

Gao YH, Liu F, Duan W, Crous PW, Cai L (2017) *Diaporthe* is paraphyletic. IMA Fungus 8(1): 153–187. https://doi.org/10.5598/imafungus.2017.08.01.11

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61(4): 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW (2013) *Diaporthe*: A genus of endophytic, saprobic and plant pathogenic fungi. Persoonia 31(1): 1–41. https://doi.org/10.3767/003158513X666844

Guarnaccia V, Crous PW (2017) Emerging citrus diseases in Europe caused by species of *Diaporthe*. IMA Fungus 8(2): 317–334. https://doi.org/10.5598/imafungus.2017.08.02.07

Guarnaccia V, Groenewald JZ, Woodhall J, Armengol J, Cinelli T, Eichmeier A, Ezra D, Fontaine F, Gramaje D, Gutierrez-Aguirregabiria A, Kaliterna J, Kiss L, Larignon P, Luque J, Mugnai L, Naor V, Raposo R, Sándor E, Váczy KZ, Crous PW (2018) *Diaporthe* diversity and pathogenicity revealed from a broad survey of grapevine diseases in Europe. Persoonia 40(1): 135–153. https://doi.org/10.3767/persoonia.2018.40.06

Guarnaccia V, Martino I, Tabone G, Brondino L, Gullino ML (2020) Fungal pathogens associated with stem blight and dieback of blueberry in northern Italy. Phytopathologia Mediterranea 59(2): 229–245. https://doi.org/10.14601/Phyto-11278

Guo YS, Crous PW, Bai Q, Fu M, Yang MM, Wang XH, Du YM, Hong N, Xu WX, Wang GP (2020) High diversity of *Diaporthe* species associated with pear shoot canker in China. Persoonia 45(1): 132–162. https://doi.org/10.3767/persoonia.2020.45.05

Harrison AL (1935) The perfect stage of *Phomopsis stewartii* on Cosmos. Mycologia 27(5): 521–526. https://doi.org/10.1080/00275514.1935.12017096

Hilário S, Amaral IA, Gonçalves MF, Lopes A, Santos L, Alves A (2020) *Diaporthe* species associated with twig blight and dieback of *Vaccinium corymbosum* in Portugal, with description of four new species. Mycologia 112(2): 293–308. https://doi.org/10.1080/00275514.2019.1698926

Huang F, Udayanga D, Wang X, Hou X, Mei X, Fu Y, Li H (2015) Endophytic *Diaporthe* associated with *Citrus*: A phylogenetic reassessment with seven new species from China. Fungal Biology 119(5): 331–347. https://doi.org/10.1016/j.funbio.2015.02.006

Huang ST, Xia JW, Zhang XG, Sun WX (2021) Morphological and phylogenetic analyses reveal three new species of *Diaporthe* from Yunnan, China. MycoKeys 78: 49–77. https://doi.org/10.3897/mycokeys.78.60878

Hyde KD, Chaiwan N, Norphanphoun C, Boonmee S, Camporesi E, Chethana KWT, Dayarathne MC, de Silva IN, Dissanayake AJ, Ekanayaka AH (2018) Mycosphere notes 169–224. Mycosphere 9(2): 271–430. https://doi.org/10.5943/mycosphere/9/2/8

Hyde KD, Dong Y, Phookamsak R, Jeewon R, Bhat DJ, Gareth Jones EB, Liu NG, Abeywickrama PD, Mapook A, Wei D (2020) Fungal diversity notes 1151–1276: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 100(1): 1–273. https://doi.org/10.1007/s13225-020-00439-5

Jiang N, Fan XL, Tian CM (2021a) Identification and characterization of leaf-inhabiting fungi from *Castanea* plantations in China. Journal of Fungi (Basel, Switzerland) 7(1): 64. https://doi.org/10.3390/jof7010064

Jiang N, Voglmayr H, Piao CG, Li Y (2021b) Two new species of *Diaporthe* (Diaporthaceae, Diaporthales) associated with tree cankers in the Netherlands. MycoKeys 85: 31–56. https://doi.org/10.3897/mycokeys.85.73107

Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 26(15): 1899–1900. https://doi.org/10.1093/bioinformatics/btq224

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Long H, Zhang Q, Hao YY, Shao XQ, Wei XX, Hyde KD, Wang Y, Zhao DG (2019) *Diaporthe* species in south-western China. MycoKeys 57: 113–127. https://doi.org/10.3897/mycokeys.57.35448

Miller MA, Pfeiffer W, Schwartz T (2012) The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment. Bridging from the extreme to the campus and beyond. Association for Computing Machinery, USA, 8 pp. https://doi.org/10.1145/2335755.2335836

Nitschke T (1870) Pyrenomycetes Germanici (2nd edn.). Eduard T rewendt, Breslau, 161–320.

Norphanphoun C, Gentekaki E, Hongsanan S, Jayawardena R, Senanayake C, Manawasinghe I, Abeywickrama P, Bhunjun CS, Hyde KD (2022) *Diaporthe*: Formalizing species-group concepts. Mycosphere 13(1): 752–819. https://doi.org/10.5943/mycosphere/13/1/9

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7(1): 103–116. https://doi.org/10.1006/mpev.1996.0376

Petrović K, Riccioni L, Đorđević V, Balešević-Tubić S, Miladinović J, Ćeran M, Rajković D (2018) *Diaporthe pseudolongicolla*: The new pathogen on soybean seed in Serbia. Ratarstvo i Povrtarstvo 55(2): 103–109. https://doi.org/10.5937/ratpov55-18582

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rossman AY, Farr DF, Castlebury LA (2007) A review of the phylogeny and biology of the *Diaporthales*. Mycoscience 48(3): 135–144. https://doi.org/10.1007/S10267-007-0347-7

Rossman AY, Adams GC, Cannon PF, Castlebury LA, Crous PW, Gryzenhout M, Jaklitsch WM, Mejia LC, Stoykov D, Udayanga D, Voglmayr H, Walker DM (2015) Recommendations of generic names in *Diaporthales* competing for protection or use. IMA Fungus 6(1): 145–154. https://doi.org/10.5598/imafungus.2015.06.01.09

Santos JM, Phillips AJL (2009) Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. Fungal Diversity 34(11): 111–125.

Santos JM, Correia VG, Phillips AJL (2010) Primers for mating-type diagnosis in *Diaporthe* and *Phomopsis*, their use in teleomorph induction in vitro and biological species definition. Fungal Biology 114(2–3): 255–270. https://doi.org/10.1016/j.funbio.2010.01.007

Santos JM, Vrandečić K, Ćosić J, Duvnjak T, Phillips AJL (2011) Resolving the *Diaporthe* species occurring on soybean in Croatia. Persoonia 27(1): 9–19. https://doi.org/10.3767/003158511X603719

Senanayake IC, Crous PW, Groenewald JZ, Maharachchikumbura SSN, Jeewon R, Phillips AJL, Bhat DJ, Perera RH, Li QR, Li WJ, Tangthirasunun N, Norphanphoun C, Karunarathna SC, Camporesi E, Manawasighe IS, Al-Sadi AM, Hyde KD (2017) Families of *Diaporthales* based on morphological and phylogenetic evidence. Studies in Mycology 86(1): 217–296. https://doi.org/10.1016/j.simyco.2017.07.003

Senanayake IC, Jeewon R, Chomnunti P, Wanasinghe DN, Norphanphoun C, Karunarathna A, Pem D, Perera RH, Camporesi E, McKenzie EHC, Hyde KD, Karunarathna SC (2018) Taxonomic circumscription of *Diaporthales* based on multigene phylogeny and morphology. Fungal Diversity 93(1): 241–443. https://doi.org/10.1007/s13225-018-0410-z

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sun W, Huang S, Xia J, Zhang X, Li Z (2021) Morphological and molecular identification of *Diaporthe* species in south-western China, with description of eight new species. MycoKeys 77: 65–95. https://doi.org/10.3897/mycokeys.77.59852

Thompson SM, Tan YP, Young AJ, Neate SM, Aitken EAB, Shivas RG (2011) Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe* (*Phomopsis*) species. Persoonia 27(1): 80–89. https://doi.org/10.3767/003158511X617110

Thompson SM, Tan YP, Neate SM, Grams RM, Shivas RG, Lindbeck K, Aitken EAB (2018) *Diaporthe novem* isolated from sunflower (*Helianthus annuus*) and other crop and weed hosts in Australia. European Journal of Plant Pathology 152(3): 823–831. https://doi.org/10.1007/s10658-018-1515-7

Udayanga D, Liu X, McKenzie EH, Chukeatirote E, Bahkali AH, Hyde KD (2011) The genus *Phomopsis*: Biology, applications, species concepts and names of common phytopathogens. Fungal Diversity 50(1): 189–225. https://doi.org/10.1007/s13225-011-0126-9

Udayanga D, Liu X, Crous PW, McKenzie EH, Chukeatirote E, Chukeatirote E, Hyde KD (2012a) A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). Fungal Diversity 56(1): 157–171. https://doi.org/10.1007/s13225-012-0190-9

Udayanga D, Liu X, McKenzie EH, Chukeatirote E, Hyde KD (2012b) Multi-locus phylogeny reveals three new species of *Diaporthe* from Thailand. Cryptogamie. Mycologie 33(3): 295–309. https://doi.org/10.7872/crym.v33.iss3.2012.295

Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2014) Insights into the genus *Diaporthe*: Phylogenetic species delimitation in the *D. eres* species complex. Fungal Diversity 67(1): 203–229. https://doi.org/10.1007/s13225-014-0297-2

Udayanga D, Castlebury LA, Rossman AY, Hyde KD (2014a) Species limits in *Diaporthe*: Molecular re-assessment of *D. citri*, *D. cytosporella*, *D. foeniculina* and *D. rudis*. Persoonia 32(1): 83–101. https://doi.org/10.3767/003158514X679984

Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2014b) Insights into the genus *Diaporthe*: Phylogenetic species delimitation in the *D. eres* species complex. Fungal Diversity 67(1): 203–229. https://doi.org/10.1007/s13225-014-0297-2

Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2015) The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. Fungal Biology 119(5): 383–407. https://doi.org/10.1016/j.funbio.2014.10.009

van der Aa HA, Noordeloos ME, de Gruyter J (1990) Species concepts in some larger genera of the *Coelomycetes*. Studies in Mycology 32: 3–19.

van Rensburg JCJ, Lamprecht SC, Groenewald JZ, Castlebury LA, Crous PW (2006) Characterization of *Phomopsis* spp. associated with dieback of rooibos (*Aspalathus linearis*) in South Africa. Studies in Mycology 55: 65–74. https://doi.org/10.3114/sim.55.1.65

Vrandecic K, Cosic J, Jurkovic D, Riccioni L, Duvnjak T (2007) First report of *Phomopsis longicolla* on cocklebur (*Xanthium strumarium*) in Croatia. Plant Disease 91(12): 1687–1687. https://doi.org/10.1094/PDIS-91-12-1687B

Vrandecic K, Jurkovic D, Riccioni L, Cosic J, Duvnjak T (2010) *Xanthium italicum*, *Xanthium strumarium* and *Arctium lappa* as new hosts for *Diaporthe helianthi*. Mycopathologia 170(1): 51–60. https://doi.org/10.1007/s11046-010-9289-2

Vrandecic K, Jurkovic D, Cosic J, Postic J, Riccioni L (2011) First report of cane blight on blackberry caused by *Diaporthe eres* in Croatia. Plant Disease 95(5): 612–612. https://doi.org/10.1094/PDIS-11-10-0860

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18: 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wrona CJ, Mohankumar V, Schoeman MH, Tan YP, Shivas RG, Jeff-Ego OS, Akinsanmi OA (2020) *Phomopsis* husk rot of macadamia in Australia and South Africa caused by novel *Diaporthe* species. Plant Pathology 69(5): 911–921. https://doi.org/10.1111/ppa.13170

Yang Q, Fan XL, Guarnaccia V, Tian CM (2018) High diversity of *Diaporthe* species associated with dieback diseases in China, with twelve new species described. MycoKeys 39: 97–149. https://doi.org/10.3897/mycokeys.39.26914

Yang Q, Jiang N, Tian CM (2020) Three new *Diaporthe* species from Shaanxi Province, China. MycoKeys 67: 1–18. https://doi.org/10.3897/mycokeys.67.49483

Yang Q, Jiang N, Tian CM (2021) New species and records of *Diaporthe* from Jiangxi Province, China. MycoKeys 77: 41–64. https://doi.org/10.3897/mycokeys.77.59999

Zhang QM, Yu CL, Li GF, Wang CX (2018) First report of *Diaporthe eres* causing twig canker on *Zizyphus jujuba* (Jujube) in China. Plant Disease 102(7): 1458. https://doi.org/10.1094/PDIS-12-17-1910-PDN